2010

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The Journal of Immunology

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J Immunol 2010; 184:844-850; Prepublished online 14 December 2009;
doi: 10.4049/jimmunol.0902364
http://www.jimmunol.org/content/184/2/844

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852

Print ISSN: 0022-1767 Online ISSN: 1550-6606.

The Strength of B Cell Interaction with Antigen Determines the Degree of IgM Polymerization

Jianmin Ye,* Erin S. Bromage,*,[1] and Stephen L. Kaattari*

The induction of variable disulfide polymerization of IgM in the trout (*Oncorhynchus mykiss*) and its effect on its half-life were examined. An association between greater Ab affinity and increased disulfide polymerization was first indicated by the observation of this increased IgM disulfide polymerization during the process of affinity maturation. A direct association between Ab affinity and disulfide polymerization was then established by the fractionation of individual sera into high- and low-affinity subpopulations, which also resulted in the partitioning of high and low degrees of disulfide polymerization. The ability of high-affinity B cells to produce more highly polymerized Abs upon Ag induction was demonstrated by in vitro Ag-driven selection. Low Ag concentrations, which elicited only high-affinity Abs, also possessed the highest degree of polymerization, whereas higher concentrations of Ag elicited a broader array of Ab affinities, yielding a lower average affinity and degree of polymerization. Half-life studies revealed that the high-affinity, highly polymerized Abs possessed longer half-lives than the lower-affinity, lightly polymerized Abs. Finally, although the affinity for Ag is associated with elevated levels of polymerization, analysis of naive Ig revealed that the degree of polymerization alone, not affinity, appears sufficient to prolong Ig half-life. ***The Journal of Immunology*****, 2010, 184: 844–850.**

Variability in the degree of interheavy chain disulfide polymerization within the tetrameric teleost IgM molecule has been observed in >15 species (1–7). This form of structural diversity, which has also been reported in rat IgA (8) and human IgG_4 (9), can be easily observed when performing denaturing, nonreducing electrophoresis. Under these conditions whole native tetramers (teleost IgM), dimers (rat IgA), or monomers (human IgG_4) can be resolved into smaller disulfide polymerized subunits (1–9). In contradistinction with past work on mammalian IgM (10), it would appear that stringency in the complete disulfide polymerization of IgM is obviously not as critical for teleosts as it may be in mammals. The mechanisms giving rise to this structural diversity, as well as the functional implications, are yet to be addressed (11). Although other teleost isotypes have been identified including an IgD (12, 13), and a novel IgZ/IgT (14, 15), IgM represents the primary systemic and the likely secretory (16) Ig in teleosts. Past transgenic expression studies have demonstrated that the C_μ gene can give rise to all the isoforms observed in vivo (17), thus this diversity is not due to isotypy. In addition, two-dimensional electrophoretic analysis of immunopurified Abs demonstrated the in vivo clonal expression of all redox (2) isoforms. Thus, expression of this isoform diversity must occur post-translationally within each plasma cell or plasmablast. Although different teleost species appear to produce IgM with different ratios of the polymerized subunits, quantitatively different ratios had not been reported within individual fish until our simultaneous structural analyses of mucosal and systemic Abs (16). In that case, mucosal IgM appeared to demonstrate considerably less disulfide polymerization than did serum IgM. Most recently, we discovered that late immune serum Abs appeared to possess Abs with greater degrees of polymerization than did Abs retrieved earlier in the response from the same individuals. This latter observation suggested the possibility that higher affinity Abs may also possess a greater degree of disulfide polymerization, as the process of affinity maturation should increase the relative concentration of high-affinity Abs late in the response (18). Thus, we undertook a rigorous examination of the possible association of Ab affinity with disulfide polymerization with trout IgM.

Materials and Methods

Animals

Rainbow trout (*Onchorhyncus mykiss*) were obtained from Troutlodge (Sumner, WA) and maintained in a recirculating system as described previously (19). All animal protocols were reviewed and approved by the Institutional Animal Care and Use Committee of the College of William and Mary.

Ag preparation and immunization

Trinitrophenylated keyhole limpet hemocyanin (TNP-KLH), the immunogen for in vivo affinity maturation studies, was produced following previously published methods (16), as was TNP-BSA, the Ag used for affinity and titer determinations, except that the latter was prepared with concentrations of picrylsulfonic acid (Sigma-Aldrich, St. Louis, MO) adjusted so that the average molar haptenation ratios achieved were TNP_1-BSA or TNP_{13}-BSA. These latter hapten-protein conjugates were used for immunoadsorption upon linking to cyanogen bromide-activated sepharose beads (Sigma-Aldrich), per the manufacturer's instructions. TNP-LPS (immunogen for in vitro Ab induction studies) was prepared as previously described (20) using LPS from *Escherichia coli* O55:B5 (Sigma-Aldrich). Trout (200–300 g) that were used in the selective immunopurification and affinity maturation experiments were immunized by i.p. injection with 100 μg TNP-KLH in a total volume of 0.2 ml emulsified 1:1 in Freund's complete adjuvant: saline. At regular postimmunization intervals, sera were prepared from these fish as previously described (16). Peripheral blood lymphocytes used for in vitro Ag stimulation experiments were derived from fish immunized by i.p. injection with 100 μg TNP-LPS in

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Received for publication July 21, 2009. Accepted for publication November 10, 2009.

This work was supported by National Research Initiative Grants 2002-35204-11685 and 2005-01594-1671 from the United States Department of Agriculture Cooperative State Research, Education, and Extension Service.

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Abbreviations used in this paper: PI, polymerization index; pIgR, polymeric Ig receptor; PTM, post-translational modification; SDS, sodium dodecyl sulfate; TNP-BSA, trinitrophenylated bovine serum albumin; TNP-KLH, trinitrophenylated keyhole limpet hemocyanin; TNP-LPS, trinitrophenylated lipopolysaccharide.

www.jimmunol.org/cgi/doi/10.4049/jimmunol.0902364

a total volume of 0.2 ml emulsified 1:1 in Freund's complete adjuvant: saline 20 wk before leukocyte procurement.

Competitive immunoadsorption

High-affinity Abs were isolated from individual anti-TNP sera by incubation of an excess of antiserum with a limiting quantity of TNP_1-BSA conjugated sepharose beads (4-fold the binding capacity of the bead aliquot). These incubations were performed overnight at 4°C in a Bio-Spin chromatography column (Bio-Rad, Hercules, CA) using a rotator. After this incubation, the column was washed with ten bed-volumes of PBS. The unbound protein fraction was then incubated with an excess of TNP_{13}-BSA beads to retrieve all unbound (lower affinity) Abs. Controls containing all Abs were prepared by incubating 1000 units (21) of the antisera with an excess of TNP_{13}-BSA beads, thereby securing 100% adsorption for comparison with the high- versus low-affinity fractions from the same antiserum. All adsorbed Abs were displaced from the immunosorbent with 10^{-3} M TNP-lysine in PBS. All eluates were extensively dialyzed against PBS to remove the hapten and then concentrated with polyethylene glycol 20,000 (Sigma-Aldrich) before affinity analysis (21). Affinity profiles of the purified Abs were determined by the use of an ELISA affinity-partitioning methodology originally devised by Neito et al. (22) and modified by Shapiro et al. (21).

Cell culture

Trout PBL isolation and culture was conducted as previously described (19) with the following modification: to avoid compromising affinity analyses of the in vitro generated Abs by residual Ag, all lymphocyte cultures were induced by pulsing with the TNP-LPS Ag for 3 d. On day 3, the cultures were washed four times and the media replaced by tissue culture medium without Ag. On day 7 of culture, supernatants were harvested and anti-TNP Abs were immunopurified using TNP_{13}-BSA beads and analyzed as described above.

Polymerization index determination

The degree of Ig disulfide polymerization within the IgM tetramers was ascertained by densitometric analysis of electrophoretically isolated (denaturing, nonreducing condtions) IgM subunits as previously described (16) with the following modifications. After transfer, the membranes were blocked with 4.0% (w/v) casein in 0.1% (Tween 20) TBST blocking buffer. The membranes were then incubated with 0.5 μg/ml anti-trout H chain mAb 1–14 (23). This anti-trout IgM reagent was capable of adsorbing all anti-TNP reactivity from the serum, and renders a signal directly proportional to the Ab protein regardless of the redox structure (24). The membrane was then washed and incubated in 0.4 μg/ml AlexaFluor 680 goat anti-mouse IgG (H + L) (Invitrogen-Molecular Probes, Eugene, OR). To ascertain the degree of intermonomeric disulfide bonding, quantitative densitometry of the bands corresponding to (a) fully polymerized tetramers, (b) trimeric and (c) dimeric subunits, and (d) total IgM was performed using the Odyssey Infrared Imaging System (LI-COR Biosciences, Lincoln, NE; Fig. 1*A*, 1*B*). A quantitative assessment of the average number of intermonomeric disulfides (polymerization index [PI]) per IgM was derived by the following formula:

$$\mathrm{PI} = [(a \times 3.5) + (b \times 2) + (c \times 1)] / d$$

where a is (relative molar concentration of tetramers) = OD of disulfide linked tetramers/800 kDa; b is (relative molar concentration of trimeric subunits) = OD of disulfide linked trimers/600 kDa; c is (relative molar concentration of dimeric subunits) = OD of disulfide linked dimmers/400 kDa; d is (relative molar concentration of all IgM) = sum of all OD of all Ig bands/800 kDa.

Using the data depicted in Fig. 1 for the low (*a*) and high (*b*) affinity Abs, we would obtain the following PIs:

$$\mathrm{PI_a} = \{[(60.1 / 800) \times 3.5] + [(80.5 / 600) \times 2] + [(58.4 / 400 \times 1)]\} / (275.8 / 800) = 1.97$$

$$\mathrm{PI_b} = \{[(93.5 / 800) \times 3.5] + [(112.9 / 600) \times 2] + [(44.9 / 400) \times 1)]\} / (289.3 / 800) = 2.48.$$

The coefficients in the PI formula are equivalent to the number of intermonomeric disulfides to be found within each fully linked tetramer (3.5), trimeric (2) and dimeric (1) subunit. A value of 3.5 is used for the cross-linked tetramer, because it can be either fully cyclic (four disulfides) or linear (three disulfides); as yet, we are unable to distinguish between these two forms.

Half-life analyses

The Abs used for the half-life study were derived from an antisera pool obtained from fish previously immunized with TNP-KLH over 5, 10, and 15 wk. This temporally composite pool insured a wide variety of affinities and degrees of polymerization within the transferred Abs. Approximately 100,000 units (21) of immunopurified anti-TNP Ab (~100 μg) were transferred into anesthetized naive hosts via cardiac injection. At 5 min (day 0) postinjection, and daily thereafter, 100 μl blood (<1% of total blood volume) was withdrawn from the caudal vessel. Plasma was collected and frozen until analyzed. The specific anti-TNP titer and affinity of the plasma samples were assessed via ELISA and the affinity-partitioning method (21), respectively. All concentrations of purified Abs were adjusted to account for any blood volume (25) differences. The retrieved TNP-specific Ab was immunopurified and analyzed by sodium dodecyl sulfate (SDS)-composite agarose/polyacrylamide gel electrophoresis (16) electrophoresis, and the Ab PIs were quantified as described above. IgM from a pool of naive fish was isolated using Sephacryl HR-S-300 gel filtration (General Electric, Fairfield, CT). The product was then assessed for purity via SDS-polyacrylamide gel electrophoresis and gel filtration. Nonimmune (naive) IgM was biotinylated with sulfo-NHS-biotin (Sigma-Aldrich) per the manufacturer's directions. As previously described, the purified and biotinylated trout IgM was injected into anesthetized naive hosts via cardiac injection. The concentrations of biotinylated Ig were determined by an avidin capture ELISA wherein avidin (Sigma-Aldrich) was coated on ELISA plates at a concentration of 0.2 μg/ml in bicarbonate coating buffer. Dilutions of the recipient sera were then incubated on the avidin coated wells for 1 h. Plates were subsequently developed using avidin-HRP with an ABTS substrate solution (21). Initial reaction rates were calculated and plotted to determine the relative mounts of biotinylated activity per ml of the original sera (21). PIs were determined using stained SDS-composite agarose/polyacrylamide gel electrophoresis gels of electrophoresed IgM using AlexaFluor 680 streptavidin (Invitrogen-Molecular Probes).

Statistical analysis

All data in graphs and tables are presented as mean ± SEM. The correlation between Ab affinity and the degree of disulfide polymerization (polymerization coefficient) was estimated by linear regression. The statistical significance of the half-life differences were evaluated by unpaired Student *t* tests with two-tailed distributions. Values of $p < 0.05$ were considered significant.

Results

Affinity maturation results in increased disulfide polymerization

Based on the preliminary observation that trout exhibited lower PIs in Abs derived early in an immune response than those procured later, it was hypothesized that the degree of polymerization might be linked to the affinity of the Ab. Thus, as fish undergo affinity maturation (18), their Abs might undergo a parallel increase in polymerization. Therefore, a group of five fish was immunized with TNP-KLH, and titers and affinities to TNP were monitored at weeks 0, 5, 10, 15, 20, and 27 postimmunization (Fig. 2*A*). It can be seen that the anti-TNP affinities increased at every time point through week 20, 5 wk past the point at which the highest titers were observed. Abs from these sera were then adsorbed on TNP_{13}-BSA beads and structurally analyzed (Fig. 2*B*). In one representative example (Fig. 2*C*) it can be seen that, as the average affinity increased over time, so did the proportion of heavily polymerized tetramers, while lightly polymerized IgM Abs (possessing monomers) decreased in concentration. All individuals demonstrated a parallel increase in PI as the affinity increased over the 27-wk period.

The relationship between Ab affinity and disulfide polymerization within individual sera

It was reasoned that if the degree of polymerization were strictly dependent upon the affinity of the Abs, then fractionation of individual antisera into high and low affinity subpopulations should reveal a corresponding association with the degree of polymerization. Three individual antisera were, therefore, affinity-fractionated

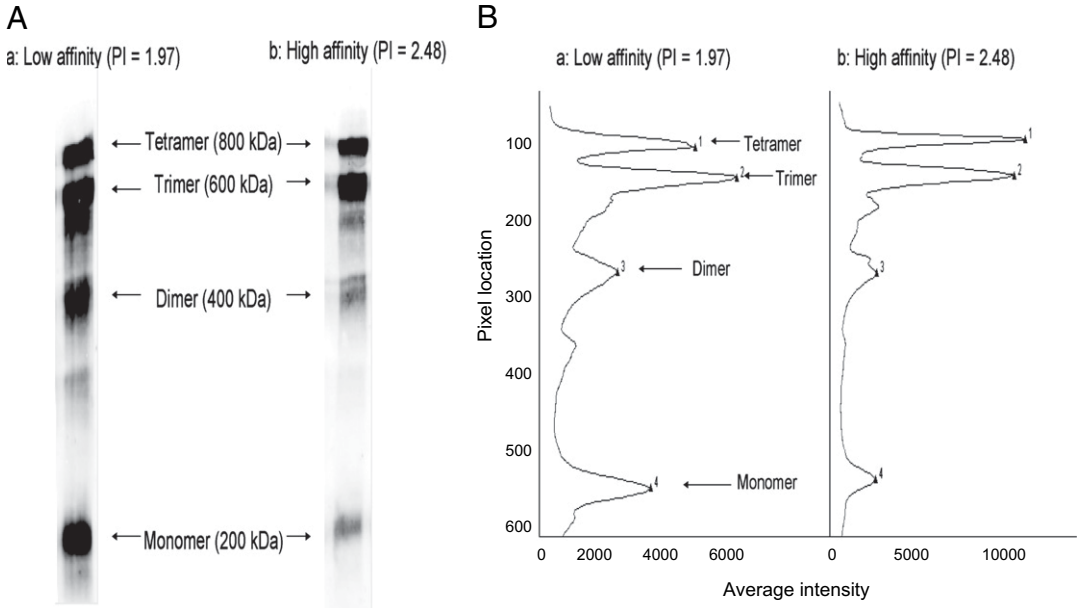

FIGURE 1. PI analysis. *A*, Immunoadsorbed aliquots of IgM from early (low affinity) and late (high affinity) antisera from a TNP-KLH immunized trout underwent electrophoresis under denaturing, nonreducing conditions in a composite acrylamide-agarose gel, and then immunoblotting. *B*, Quantitative densitometry was performed on the Ig protein bands transferred to Immobilon-FL polyvinylidene difluoride membrane (Millipore, Bedford, MA) and developed using anti-trout H chain Ab 1-14/AlexaFluor680 goat anti-mouse IgG. Analysis of stained band intensity was quantified on an Odyssey Infrared Imaging System (LI-COR, Lincoln, NE).

into high- and low-affinity pools by competitive immunoadsorption. To accomplish this, a limiting amount of a TNP_1-BSA adsorbent, capable of adsorbing only 25% of the total Ab activity (10,000,000 units) contained in an aliquot of antiserum, was used to procure high-affinity Abs. The remaining lower affinity Abs (~75% of the total) were retrieved by complete adsorption upon a highly substituted (TNP_{13}-BSA) adsorbent. These immunopurified Abs were then compared with one another and the total Ab pool (Fig. 3). Affinity analysis demonstrated the success of isolating these affinity subpopulations (Fig. 3*A*). The PI of each purified Ab pool was then determined. These indices demonstrated that the higher affinity subpopulations were more heavily polymerized (Fig. 3*B*).

In vitro Ag-driven selection demonstrates a differential level of polymerization based on affinity for the inducing Ag

As the above analyses were performed on serum Abs, the mechanism responsible for producing this association between affinity and polymerization could not be directly ascertained. For example,

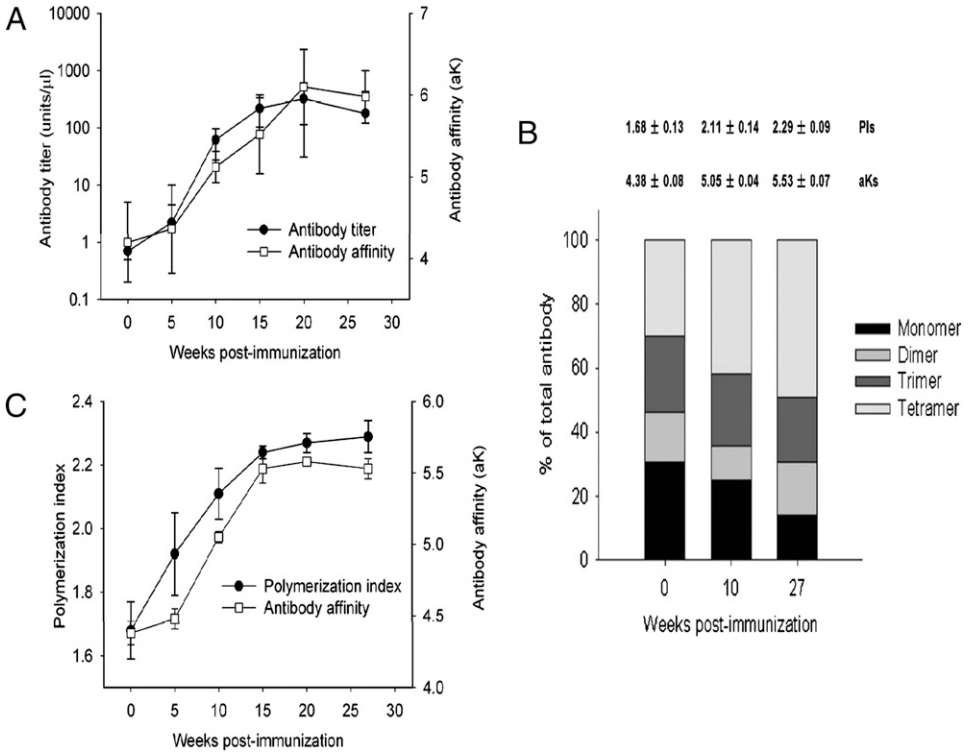

FIGURE 2. Increase in IgM affinity after immunization (affinity maturation) is paralleled by increased polymerization. *A*, Five trout were immunized with TNP-KLH and periodically bled, titered, and assessed for Ab affinity. Error bars represent the SEM of five individuals at each time point. *B*, Denaturation of the native tetrameric Ab reveals the changing degree of polymerization yielding differing ratios of tetrameric, trimeric, dimeric, or monomeric subunits at 0, 10, and 27 wk post-immunization (as a percentage the total Ab protein). *C*, The PIs demonstrate a parallel increase of affinity with polymerization over time. Error bars denote SEM for five individuals at each time point.

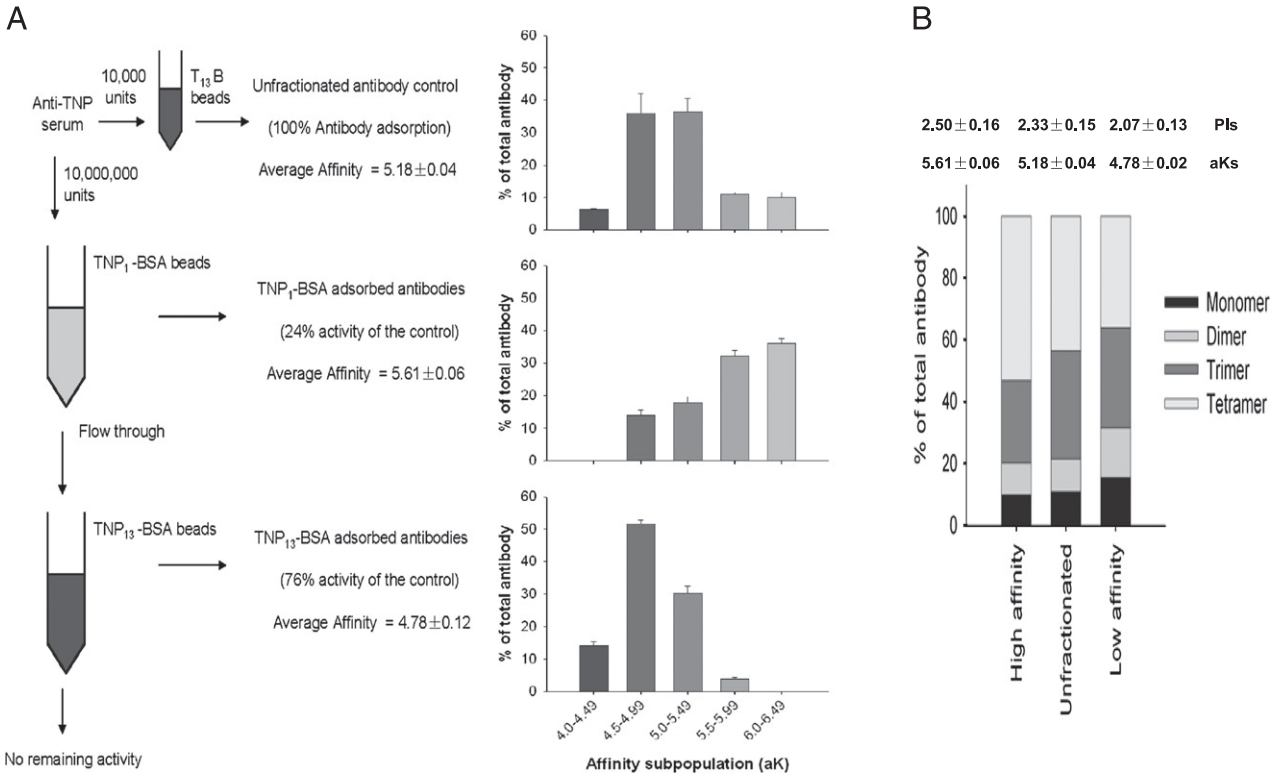

FIGURE 3. Affinity fractionation of serum IgM Abs. *A* (left), Limiting amounts of TNP_1-BSA immunosorbent beads were used to extract the high-affinity Abs from aliquots of anti-TNP sera collected from TNP-KLH–immunized animals as depicted in the schema provided. Unbound serum Abs from this adsorbent were then retrieved using an excess of TNP_{13}-BSA beads. *A* (right), The average affinity profiles from three original antisera as well as their high and low affinity fractions are depicted. Error bars denote SEM for three analyses. *B*, The proportionate shifts in the degree of polymerization are summarized with the average of all three affinity fractionations.

this association could be related to B cell induction and consequent IgM assembly processes. Alternatively, a post-secretory process might selectively remove or retain IgM based on structural differences. To ascertain whether the affinity for the cognate Ag could be regulating the degree of polymerization, in vitro Ag-driven selection (18) was used. Aliquots of peripheral blood lymphocytes from individual fish were stimulated with graded doses of the Ag, TNP-lipopolysaccharide (TNP-LPS), to selectively induce Abs with high versus low affinities. As such, low doses of Ag selectively elicited Ab responses from high affinity lymphocytes (Fig. 4*A*), whereas the higher doses elicited responses from a broader array of both high- and low-affinity lymphocytes, resulting in an lower average Ab affinity. All cultures were stimulated via Ag-pulsing to insure that no free Ag would be available to interact with the secreted Ab and thereby interfere with Ab affinity measurements. This Ag-driven selection of affinity was achieved, as the lowest concentrations (0.01 μg/ml) of Ag stimulated the highest affinity Abs (Fig. 4*A*). Further, these high-affinity Abs also possessed the greatest degree of polymerization, thus the selective elicitation of high-affinity interactions are associated with a greater degree of disulfide polymerization (Fig. 4*B*).

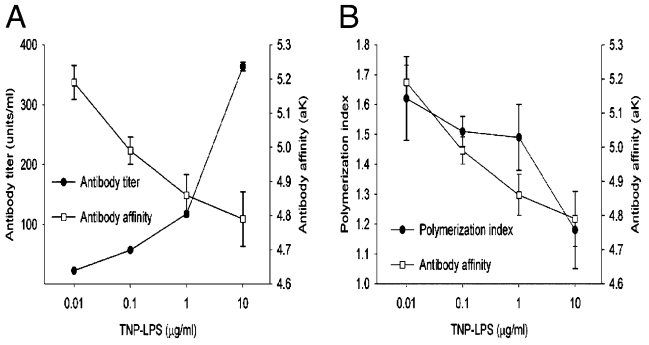

FIGURE 4. The effect of differential in vitro induction of high- versus low-affinity Abs. *A*, Aliquots of peripheral blood leukocytes from individual fish were stimulated with graded concentrations of TNP-LPS, and the resultant Ab titers and affinities were determined as depicted in this representative experiment. Error bars denote SEM for three experiments. *B*, The induced Abs from these cultures were immunopurified, and the PIs were determined and plotted together with their affinities. Error bars denote SEM for three experiments.

Relationship between disulfide polymerization and Ab/Ig half-life

Although the Ag-driven selection experiments (above) provide evidence that the degree of IgM polymerization can be explained by B cell recognition and induction, the possible role of post-secretory processes on affecting the relative concentrations of these differentially polymerized Abs was examined. This analysis was accomplished by immunopurifying anti-TNP Abs from individual donor fish, then transferring the Abs to nonimmune recipients. The Ab transfer was followed by daily bleeds (<1% of the total blood volume) of the recipients followed by immunoadsorption and analysis of the structure, affinity, and quantity of the retrieved Abs. It can be seen in Fig. 5*A* that the half-life of lightly polymerized Abs was significantly shorter than the heavily polymerized Abs ($p < 0.05$). This half-life difference resulted in an approximately half log (5-fold) increase in the average affinity of the transferred Abs within 1 wk of transfer (Fig. 5*B*; i.e., a shift in aK from 5.27 to 5.96).

The above observation prompted the query as to whether the degree of polymerization alone (i.e., independent of affinity) is sufficient to this effect on half-life. To address this, whole Ig from nonimmune fish was transferred to naive recipients. However, as the half-life of these

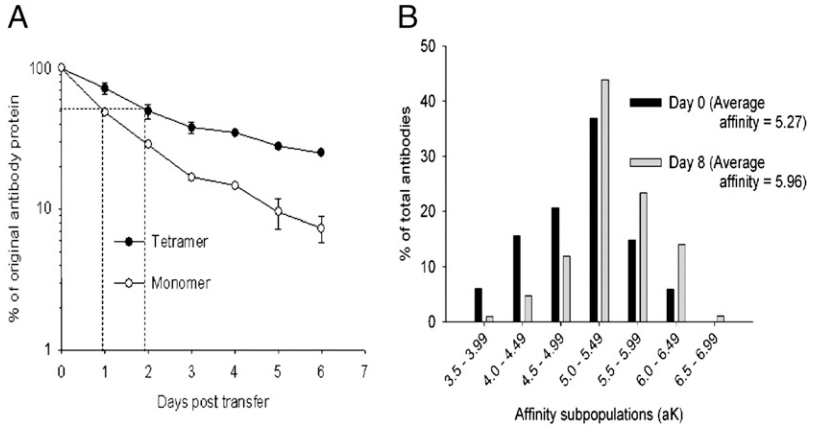

FIGURE 5. Half-life of differentially polymerized IgM Abs. *A*, Immunopurified anti-TNP Abs were transferred to naive animals. The percentage of the original cross-linked tetramers (●) and unlinked monomers (○) were determined over time. All comparisons were made with the day 0 concentration (5 min posttransfer). Estimates of each initial half-life are depicted by the dotted lines and were found to be significantly different from one another ($p = 0.05$). *B*, Immunopurified Abs from day 8 and day 0 bleeds of a representative recipient fish were assessed for their affinity distributions and average affinities.

transferred Igs could be monitored only if distinguished from the recipient's Ig, it was necessary to label the transferred Igs. We chose, therefore, to biotinylate the transferred Ig, as the process of biotinylation did not affect Ig half-life (Fig. 6*A*). Having confirmed the lack of a biotinylation effect on Ig half-life, nonimmune IgM was procured by gel filtration, biotinylated, and transferred to recipients. Monitoring the transferred nonimmune Igs once again revealed that the half-life of the lightly polymerized IgM was significantly shorter than that of the heavily polymerized tetramers (Fig. 6*B*).

Discussion

The association between affinity and disulfide polymerization within Abs from individual antisera (Fig. 3) correlated with the observed increase in PIs during affinity maturation (Fig. 2). This relationship has profound implications. The classical model of affinity maturation (26) envisions an elegantly simple process wherein a sufficiently large initial bolus of Ag can engage a wide range of BCR affinities. As the concentration of Ag diminishes, only the highest affinity B cells can bind sufficient Ag to elicit Abs. Thus, over time the average affinity of serum Abs increases. This process can be accentuated by the later development of somatic mutations, which offer an additional, de novo, repertoire of specificities, including those of even greater affinity (27). Our findings demonstrate that a significant driving force, beyond affinity maturation, results in a higher average Ab affinity in trout—that is, via the provision of an increased half-life to high-affinity Abs (Fig. 5). This increase appears to be associated with a post-translational modification of the IgM that leads to increased disulfide polymerization. To date, reports of varied polymerization correlating with Ig or protein half-life have not been forthcoming. Although Ab half-life could be directly dependent on the degree of disulfide polymerization, it is possible that this disulfide polymerization is the consequence of a more primary affinity-dependent modification that directly regulates half-life. For example, it has been observed that C-terminal, *N*-linked glycosylation affects polymeric Ig clearance (28) as well as C-terminal disulfide polymerization (29). It is also important to note that differential glycosylation could also govern other effector functions, such as opsonization of immune complexes, and complement activation (30). Therefore, if a relationship between the affinity of Ag binding, glycosylation, and polymerization exists, perhaps differential glycosylation is the underlying factor that determines the half-life differences and disulfide polymerization. Further, if glycosylation is the salient post-translational modification linking affinity to polymerization in trout, perhaps there is an unrecognized linkage between affinity and glycosylation in other vertebrate IgMs as well.

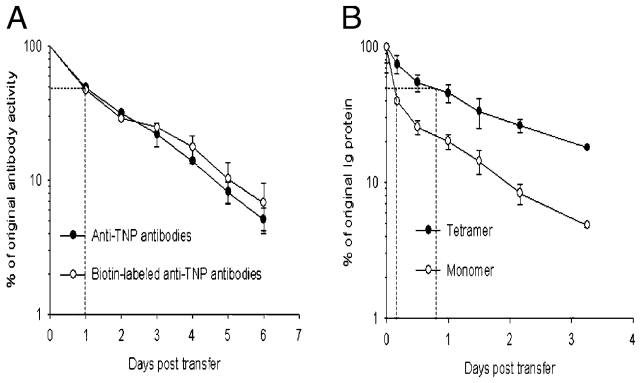

FIGURE 6. Half-life nonimmune differentially polymerized IgM. *A*, Aliquots of immunopurified anti-IgM Abs were biotinylated or left nonbiotinylated. Preparations of each were then transferred by intracardiac injection to naive recipients. Titers of each were monitored over time to independently assess the effect of biotinylation. *B*, Half-life of cross-linked tetramers versus unlinked monomers from nonimmune IgM in naive recipients. Error bars denote SEM of thre experiments. Estimation of each initial half-life is depicted by the dotted lines.

Although high-affinity, highly-polymerized Abs were observed to have longer half-lives in the absence of Ag (upon transfer to naive fish), we have yet to ascertain the effect of Ag binding on their respective half-lives. Day et al. (31) have observed that Ag binding to IgM Abs in the mouse results in the exposure of occluded high mannose residues, which could in turn accelerate complexed Ab-Ag complex removal. Therefore, if the same glycosylation effects occur in trout, the binding of Ag may equalize the removal rate of all IgM Abs. However, until Ag is bound, high-affinity Abs should have an extended persistence. Thus, the highest affinity Abs should remain in circulation longer and be available for recurrent Ag exposures.

The role of BCR affinity in controlling the degree of polymerization would seem highly probable, based on the in vitro Ag-driven selection experiments (Fig. 4). As was classically demonstrated in vivo by Goidl et al. (32), lower concentrations of Ag in vitro can also selectively elicit Abs of higher average affinities from cultures of the same lymphocyte pool (18). This was, again, demonstrated in Fig. 4*A*. More importantly, if we isolate these Abs and analyze their structure, we find that their affinity parallels the degree of polymerization (Fig. 4*B*). In this case, we are eliciting the parallel relationship from aliquots of the same cell population as opposed to examining serum Abs from different animals or from different points in time. Furthermore, as this experiment was conducted in vitro, the degree of polymerization must be caused by processes within the induced B cells only, and not a post-secretory event occurring within the tissues or circulation. Thus, the strength of BCR interaction is likely being transduced into a fine-tuned, post-

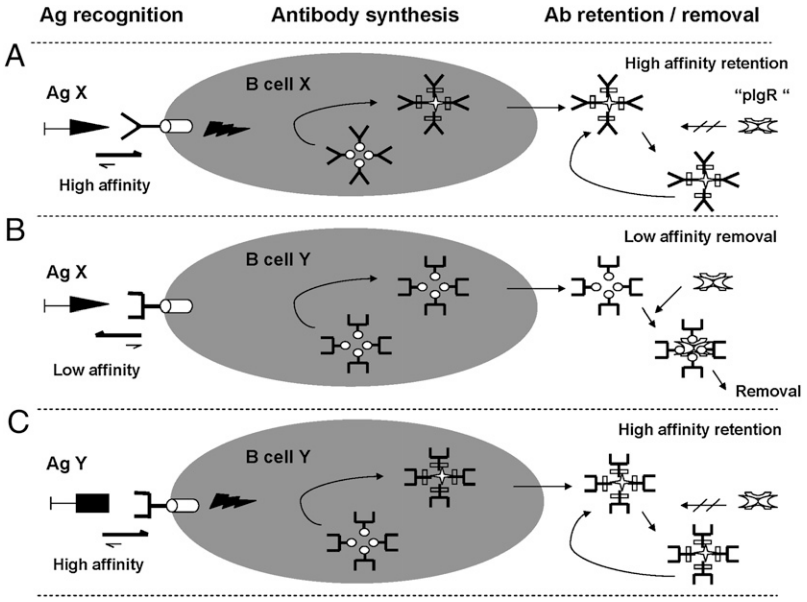

FIGURE 7. Differential post-translational modification of high- versus low-affinity Abs (a model). *A*, Ag X binds with high affinity to the BCR of B cell X. The high-affinity interaction results in a unique PTM of the Ab X (e.g., C-terminal glycosylation). This PTM results in enhanced disulfide polymerization. PTM of this Ab results in a lack of recognition and removal by circulating pIgR-like molecules upon secretion. *B*, The same Ag X interacts with B cell Y, which possesses lower affinity for the Ag. The low-affinity interaction results in less or no PTM. The unmodified Fc of Ab Y is now recognized by the pIgR-like molecules and is rapidly removed from the circulation, resulting in a significantly reduced half-life. *C*, Recognition of Ag Y, for which B cell Y possesses high affinity, results in the identical PTMs, as occurred in recognition of Ag X by B cell X. Upon secretion, Ab Y will now possess a longer half-life as did Ab X when induced by high-affinity interaction with its cognate Ag.

translational process leading to significant structural alterations of the Ab product. Although we believe that transduction of affinity into modifications of the Ab product is a novel observation, the ability of the B cell to respond differentially based on the affinity of Ag interaction is not without precedent. For example, B cell affinity thresholds (33) or ceilings (33, 34) appear to govern the degree of Ab elicitation, whereas BCR affinity can also be transduced into the differential expression of cytokines (35) or cell surface markers (35, 36) or commitment to distinct differentiation pathways (35–37).

In light of these studies, our past finding that trout mucosal IgM is significantly less polymerized than serum IgM (16) may find possible explanations. For example, it may be that 1) the mechanisms responsible for the differential half-lives of variably-polymerized IgMs are due to selective transfer or transcytosis of lightly polymerized IgM to the mucosa, 2) locally produced IgM Abs may be of consistently lower affinity, or 3) production of mucosal IgM is under different polymerization constraints than is systemic IgM. Recent work demonstrating the existence and binding of teleost polymeric Ig receptor (pIgR) to mucosal IgM (38, 39) suggests that pIgR or a pIgR-like molecule could participate in the selective removal of lightly cross-linked IgM from the circulation and delivery to the mucosa.

The C-terminal focus of IgM polymerization and glycosylation diversity in mice (40), its recognition by pIgR (41), as well as the mediation of other functions, such as mannose binding lectin recognition (42), could indicate a structural or functional nexus centering around the C-terminal disulfide (Cys 578) and proximal *N*-linked glycosylation site (Asn 573) in trout as well as mammals. However, more intriguing is the possibility that modulation of this structural/functional nexus by the strength of Ag recognition may occur in other vertebrates as in trout.

Based on the observations described above and previous work conducted by others in mammals, we have devised a possible model (Fig. 7). We propose that the high-affinity binding via the BCR is transduced into a high degree of post-translational modification (PTM) of the IgM C terminus (Fig. 7*A*). Ag-driven selection demonstrated that induction of the highest affinity cells resulted in the highest degree of disulfide polymerization. We propose that this PTM results in an easily detectable, high degree of disulfide polymerization—this modulation being easily detectable in trout because of the reliance on only the C-terminal disulfide for intermonomeric polymerization. This PTM could be due to the direct oxidation of the C-terminal disulfides or some other PTM such as C-terminal glycosylation, which then leads to the oxidized state. This PTM also acts to occlude recognition of the secreted Ab by receptors such as pIgR, which would result in its rapid removal from the circulation. However, if a B cell (via its BCR) has a lower affinity for this same Ag (Fig. 7*B*), the binding signal will not be transduced into this PTM, thus leaving the original C-terminal structure. In its unmodified form, it is readily recognized and removed from the circulation. A possible mediator of this process could be pIgR, which acts to deliver polymeric Igs to the mucosa (43) and has been identified in teleost fish (38, 39). Such a mediator could differentially remove this low-affinity, less polymerized Ig from the circulation. However, if this latter B cell encounters an Ag for which it possesses high affinity (Fig. 7*C*), PTMs will occur as in Fig. 7*A*, resulting in a loss of this pIgR-like recognition and prolonged residence within the circulation. Although we have depicted half-life being dependent on recognition and removal of unmodified Igs, it is possible that high-affinity PTMs could result in recognition and retrieval of the secreted Igs via a receptor comparable to a neonatal FcR, which acts to preserve and maintain circulating IgG concentrations (44). Either recognition and removal or retrieval mechanisms, or both sorts of mechanisms, could be used to achieve the same differential in Ig half-life among differentially modified Igs.

Acknowledgments

We thank Dr. P. Zwollo, Dr. G. Wiens, Dr. T. Harris, C. Spier, I. Kaattari, and M. Vogelbein for reading the manuscript. We also thank I. Kaattari and M. Vogelbein for technical support. This paper is Contribution No. 3048 of the Virginia Institute of Marine Science, College of William and Mary.

Disclosures

The authors have no financial conflicts of interest.

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